Designation: E388 – 04 (Reapproved 2023)

Standard Test Method for Wavelength Accuracy and Spectral Bandwidth of Fluorescence Spectrometers[1]

This standard is issued under the fixed designation E388; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the testing of the spectral bandwidth and wavelength accuracy of fluorescence spectrometers that use a monochromator for emission wavelength selection and photomultiplier tube detection. This test method can be applied to instruments that use multi-element detectors, such as diode arrays, but results must be interpreted carefully. This test method uses atomic lines between 250 nm and 1000 nm.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Summary of Test Method

2.1 The difference between the apparent wavelength and the known wavelength for a series of atomic emission lines is used as a test for wavelength accuracy. The apparent width of some of these lines is used as a test for spectral bandwidth.

3. Apparatus

3.1 *Fluorescence Spectrometer* to be tested.

3.2 *Atomic Discharge Lamps, Low-pressure,* sufficiently small to be placed in the sample cell holder of the instrument.

4. Reagent

4.1 *Scattering Suspension*—Dissolve 1 g of glycogen per litre of water, or use a dilute microsphere suspension containing 1 mL of a commercially available, concentrated microsphere suspension.

5. Procedure

5.1 The emission lines given for mercury (Hg), neon (Ne), argon (Ar), krypton (Kr), and xenon (Xe) in Table 1 are typically observable using standard commercial fluorometers, although some of them may be too weak to detect on some instruments.

5.1.1 Most fluorescence instruments will not be able to resolve very closely spaced lines such as those for Hg at 312.57 nm, 313.15 nm, and 313.18 nm, due to the relatively low resolution monochromators used in fluorescence equipment compared to those used in absorbance spectrometers. Even lower resolution fluorometers may not resolve lines separated by less than several nanometres such as those for Hg at 404.66 and 407.78, or at 576.96 and 579.07 nm.

5.1.2 In instruments using blazed grating monochromators, additional weaker lines are found due to second order diffraction of atomic lines. For instance, lines appear for Hg at 507.30 and 593.46 nm, arising from the 253.65 and 296.73 nm lines, respectively.

5.2 *Calibration and Adjustment of Emission Monochromator:*

5.2.1 With an atomic arc source properly aligned (see 5.3) in the sample cell compartment, adjust the position of the wavelength dial to give maximum signal for each of the atomic lines and record the wavelength reading. The difference between the observed value and the corresponding value in Table 1 represents the correction that must be subtracted algebraically from the wavelength reading of the instrument. The corrections may be recorded or the monochromator adjusted to give the proper values. Since there may be some backlash in

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TABLE 1 Atomic Emission Lines[A] for Wavelength Accuracy

Hg	Ne			Ar	Kr		Xe
253.65	336.99	633.44	830.03	696.54	427.40	645.63	450.10
296.73	341.79	638.30	836.57	706.72	428.30	722.41	458.28
302.15	345.42	640.11	837.76	727.29	431.96	758.74	462.43
312.57	346.66	640.22	841.72	738.40	436.26	760.15	467.12
313.15	347.26	650.65	841.84	750.39	437.61	768.53	469.70
313.18	350.12	653.29	846.34	751.47	440.00	769.45	473.42
334.15	352.05	659.90	857.14	763.51	442.52	785.48	480.70
365.02	359.35	667.83	859.13	772.38	445.39	805.95	482.97
404.66	533.08	671.70	863.46	794.82	446.37	810.44	484.33
407.78	534.11	692.95	864.70	800.62	450.24	811.29	491.65
435.84	540.06	703.24	865.44	801.48	557.03	819.00	492.32
546.07	576.44	717.39	865.55	810.37	564.96	826.32	711.96
576.96	582.01	724.52	867.95	811.53	567.25	828.10	764.20
579.07	585.25	743.89	868.19	826.45	583.29	829.81	823.16
	588.19	783.91	870.41	840.82	587.09	850.89	828.01
	594.48	792.71	877.17	842.46	599.39	877.67	834.68
	597.55	793.70	878.06	912.30	601.21	975.18	840.92
	603.00	794.32	885.39	922.45	605.61		881.94
	607.43	808.25	920.18	965.78			895.22
	609.62	811.85	930.09				979.97
	614.31	812.89	932.65				992.32
	616.36	813.64	942.54				
	621.73	825.94	948.67				
	626.65	826.61	953.42				
	630.48	826.71					

[A] Wavelength values have been obtained from Harrison, G. R., MIT Wavelength Tables, *Wavelengths by Element*, Vol 2, MIT Press, Cambridge, MA, 1982; and Zaidel, A. N., Prokofev, V. K., Raiskii, S. M., Slavnyi, V. A., and Shreider, E. Ya., *Tables of Spectral Lines*, Plenum Press, New York, NY, 1970.

the wavelength drive of scanning instruments, always approach the peak position from the same direction, if applicable.

5.2.2 When calibrating scanning-type instruments, approach the peak position in the same direction that the motor scans, if your instrument does not correct for backlash. Check the position against that recorded while scanning and, if necessary, correct as in 5.2.1.

5.3 In cases where the monochromator is designed so that a lateral displacement of the calibration source from a position directly in front of the entrance slit appears as a wavelength shift, proceed as follows:

5.3.1 Instead of placing the atomic lamp in front of the entrance slit of the monochromator, fill a sample cell with a dilute scattering suspension, as described in 4.1.

5.3.2 Place the cell in the sample position in the instrument.

5.3.3 Illuminate the cell transversely with the atomic lamp, either from the side or from above.

5.3.4 Adjust the wavelength to give the maximum signal for each of the atomic lines given in Table 1; record the wavelength reading and proceed as in 5.2.

5.4 *Adjustment of Excitation Monochromator:*

5.4.1 After the emission monochromator has been calibrated, adjust the excitation monochromator to match, as follows:

5.4.2 Place a sample cell containing either of the dilute scattering suspensions described in 4.1 in the sample cell compartment.

5.4.3 With a continuous source in the normal source position of the instrument, illuminate the suspension.

5.4.4 Set the wavelength positions of both excitation and emission monochromators at a previously determined setting used for calibration of the emission monochromator.

5.4.5 Adjust the wavelength position of the excitation monochromator to give a maximum signal, and record the wavelength reading. The difference between the observed value on the dial and the corresponding value in 5.1 represents the correction that must be subtracted algebraically from the reading of the instrumentation. The corrections may be either recorded, or the monochromator may be adjusted to give the proper value. It is possible to perform the latter using the control software for some spectrometers. As stipulated in 5.2.1, always approach the desired wavelength position in the same direction that the scan motor scans, if applicable.

5.4.6 The match of the excitation monochromator with the emission monochromator may be checked at wavelengths above or below that used in 5.4.5.

NOTE 1—Some fluorescence spectrometers are designed to allow the user to place an atomic lamp before the excitation monochromator. The lamp is installed into the instrument either by the manufacturer or by the user as specified by the manufacturer. In this case, wavelength accuracy can be calibrated for the excitation monochromator using a procedure that parallels that given in 5.2 for the emission monochromator. A scattering solution or other scattering media can then be placed at the sample position to scatter atomic lamp light into the emission monochromator to calibrate emission wavelength accuracy using a procedure that parallels that given in 5.4 for the excitation monochromator.

5.5 *Slit Width Effects:*

5.5.1 Use the narrowest practical slit widths in calibrating the wavelength scale. In cases when monochromator slits are not filled, or when intensity of fluorescence varies rapidly with wavelength, there may be an apparent wavelength error with wide slits. Under the most unfavorable conditions this error may approach one spectral bandwidth, so that narrow slits should be used for accurate wavelength measurements. As the magnitude of the error may depend on characteristics of both